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Potassium channels (ATP-regulated, calcium activated and voltage dependent potassium channels) present in inner mitochondrial membranes were implicated in cytoprotective phenomenon in various tissues. These channels modulate mitochondrial matrix volume, mitochondrial respiration and membrane potential, and generation of reactive oxygen species. In this paper we describe the biophysical and pharmacological properties of new mitochondrial potassium channels recorded in *Acanthamoeba castellanii* and potato tuber mitochondria. Additionally, properties of mitochondrial potassium channels present in neuronal, cardiac tissue and endothelial cells will be described.

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S3/7 Intramitochondrial signaling — Interactions among mitoK$_{ATP}$, PKCε, ROS, and MPT

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Our aim was to apprehend the pathways by which mitoK$_{ATP}$ opening leads to inhibition of the mitochondrial permeability transition (MPT), thereby reducing ischemia–reperfusion injury. We showed previously that mitoK$_{ATP}$ is opened by activation of a mitochondrial PKCε, designated PKCε1, that is closely associated with mitoK$_{ATP}$. MitoK$_{ATP}$ opening causes an increase in ROS production by Complex I of the respiratory chain. This ROS activates a second pool of PKCε, designated PKCε2, which inhibits the mitochondrial permeability transition (MPT). We measured mitoK$_{ATP}$-dependent changes in mitochondrial matrix volume to further investigate the relationships among PKCε, mitoK$_{ATP}$, ROS, and MPT. We present evidence that (1) H_2O_2 and NO cause mitoK$_{ATP}$ opening that is mediated by PKCε1 and not by direct actions on mitoK$_{ATP}$; (2) superoxide has no effect on mitoK$_{ATP}$ opening; (3) H_2O_2 or NO inhibits MPT opening, and both compounds do so independently of mitoK$_{ATP}$ activity via activation of PKCε2; (4) mitoK$_{ATP}$ opening induced by PKG, PMA or diazoxide is not mediated by ROS; and (5) mitoK$_{ATP}$-generated ROS activates PKCε1 and induces phosphorylation-dependent mitoK$_{ATP}$ opening *in vitro* and *in vivo*. Thus, mitoK$_{ATP}$-dependent mitoK$_{ATP}$ opening constitutes a positive feedback loop capable of maintaining the channel open after the stimulus is no longer present. This feedback pathway may be responsible for the lasting protective effect of preconditioning, colloquially known as the memory effect.

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(S3) Membrane transporters symposium abstracts (poster and raised abstracts)

S3.8 Effects of inhibitors on the unfolding of the mitochondrial ADP/ATP carrier by single-molecule force spectroscopy

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The mitochondrial ADP/ATP carrier exchanges cytosolic ADP for ATP synthesised in the mitochondrial matrix and replenishes the eukaryotic cell with metabolic energy. Two specific inhibitors of the carrier are known; atractyloside (ATR) and carboxyatractyloside (CATR), which differ in one carboxylate. Reconstituted histidine-tagged yeast ADP/ATP carrier AAC3 with either ATR or CATR bound was subjected to single-molecule force spectroscopy. The amino-terminal end of the protein was pulled out of the α-helical bundle in pairs of helices, reflecting the tripartite structure of the carrier. Additional resistance to unfolding was observed on helix H2 when CATR was bound rather than ATR. Two-dimensional NMR spectroscopy was used to confirm the stereochemistry of ATR, showing that the additional carboxylate of CATR is in the equatorial position. We interpret the extra resistance to be caused by the removal of the inhibitor together with the first two α-helices of the carrier, as the inhibitor is bound most strongly to these α-helices. The single-molecule force spectroscopy studies explain why CATR confers additional structural stability to the carrier.

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S3.9 Effect of single gene deletions of *mrpA–G* and *mrpE* point mutations on activity of the Mrp Na^+/H^+ antiporter of alkaliphilic *Bacillus* and formation of hetero-oligomeric Mrp complex

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The putative "multi-subunit" Mrp family of secondary monovalent cation proton antiporters is physiologically important in diverse bacteria. The aim of this study was to examine structure–function of the product of the seven-gene *mrp* operon from an alkaliphilic *Bacillus*. The cloned operon was engineered so that each of the Mrp proteins (MrpA–G) could be detected. When expressed in an antiporter-deficient strain of *Escherichia coli*, Mrp-dependent $Na^+(Li^+)/H^+$ antiport was observed. Analyses by combined Blue Native electrophoresis and SDS-PAGE demonstrated complexes that contain all 7 gene products in size ranges that could be monomers and dimers. Analyses of single, nonpolar *mrp* gene deletion mutants showed that: all Mrp proteins were required for significant antiport activity; MrpD is required for stable membrane incorporation of all other Mrp proteins;